Rapid Compositional Analysis of 61 *Zea mays* Samples Using Near-infrared Spectroscopy

Jonathan Meuser

Department of Energy Energy Research Undergraduate Laboratory Fellowships
National Science Foundation
Colorado State University
National Renewable Energy Laboratory
Golden, Colorado, 80401

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Abstract

Rapid Compositional Analysis of 61 *Zea mays* Samples Using Near-infrared Spectroscopy. JONATHAN MEUSER (University of California, Davis, California 95616), STEVEN THOMAS and TAMMY HAYWARD (National Renewable Energy Laboratory, Golden, Colorado, 80401).

A major challenge in commercializing ethanol production from corn stover is the great variability in composition of commonly grown varieties. Only when the variables that determine stover composition are isolated can optimum stover be produced, making possible consistent process yields and economics. The extent to which environmental and genetic factors affect cell wall composition in corn stover is unknown. In this study, the cell-wall composition of 61 stover samples was determined by near-infrared spectroscopy (NIR). With NIR, the composition of many samples can be economically, accurately and quickly determined, providing the bulk of data necessary to perform meaningful statistics. As we approach a high-throughput system of compositional analysis, outlying samples and the variables that cause their dissimilarity may be more readily understood. For instance, though closely related to commercial corn, *Teosinte* parviglumis drastically differed in composition. Also, Pioneer B73xMo17 and Pioneer 33P67 were tested to determine the affect of irrigation, planting density and variety on cell-wall composition. These variables proved to be insignificant factors in composition, however, 33P67 grown under the same conditions had unusual variability in soluble sugars. Because irrigation and planting density commonly differs between fields, eliminating these two variables as factors affecting composition allows greater flexibility in growing and experimentating on high value stover. Further research into the exact cause of *Teosinte's* structural differences may illuminate genetic causes of cell-wall variation for all corn.

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School Author Attends: University of California at Davis

DOE National Laboratory Attended: National Renewable Energy Lab

Mentor's Name: Dr. Steven Thomas

Phone: (303) 384-7775

e-mail Address: thomas steven@nrel.gov

Presenter's Name: Jonathan Meuser
Mailing Address: 850 Adams St. "B"
City/State/ZIP: Davis, CA 95616
Phone: (530) 219-2483

e-mail Address: jemeuser@ucdavis.edu

Is this being submitted for publication?

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Introduction

Corn stover - the stalk, leaves and cobs of corn - has had few traditional uses, and it is usually burned or turned back into the soil. Contemporary experimentation on stover feedstock for ethanol fuel production has promoted a fresh interest in the composition of corn stover, however. According to the U.S. Department of Energy (DOE), "U.S. farmers plant about 80 million acres of corn each year, with a potential (wasted) stover harvest of some 120 million dry tons." Available corn stover thus represents the most abundant bioenergy feedstock available now. Accordingly, the DOE has set a goal of having nine corn-stover-fed commercial ethanol plants in operation by 2006.

Inconsistent conversion of corn stover to ethanol caused by compositional variation thwarts this goal. Consisting primarily of cellulose, hemicellulose and lignin, commercial corn stover diversity highly affects its performance as a fermentable feedstock. One study suggested the carbohydrate content variation in modern corn stover translates into a minimum ethanol selling price difference of 20 cents/gallon (Thomas et. al. 2001). Likewise, optimization of the production process in both pretreatment and simultaneous saccharification and fermentation (SSF) has been complicated by such variations in stover composition, making it difficult to estimate the cost of production. Identifying the factors that generate quality stover feedstock for ethanol production may benefit farmers and/or seed companies in producing a higher quality stover for ethanol production, making ethanol fuel a cost-effective alternative to fossil fuels.

In this study, we compared the composition of many genetically distinct lines, both commercial and exotic, grown under a range of environmental conditions to explain stover compositional variations.

Materials and Methods

In November 2001, Ken Russell, Assistant Professor at the University of Nebraska, Lincoln, collected residual corn stover, including leaves and stalk, from 44 plots of corn representing 21 varieties from two fields. He dried them to below 20% moisture, and shipped them individually packed by UPS ground delivery to the National Renewable Energy Lab (NREL). On arrival, all samples were air-dried for 3 days at 80°C in the NREL Field Test Laboratory Building greenhouse. All the samples were photographed, indexed for dryness, and given an identification number. The samples were professionally milled 6 months later into a coarsely ground homogenous mixture that passed through a ¼-in. screen. Each milled sample was divided into equivalent 500 g portions and stored in large, labeled plastic bags placed inside plastic buckets, also labeled.

We collected near-infrared (NIR) reflectance spectral data in duplicate using the FOSS NIR Forage spectrometer (FOSS NIR systems, Inc., Silver Spring, Maryland) and WinISI analytical software. Representative grabs were loaded into a clean natural product cell, compressed against the quartz lens with the sample cell backing, and placed into the NIR spectrometer for analysis.

The percentage of dry weight of 12 corn stover constituents were determined using a model developed at the NREL Biotechnology Center for Fuels and Chemicals. Nothing can be proven by variation below the method error of the model. Because +/-1.5% is the method error of near-infrared prediction based on wet chemical analysis, constituents with a range in percentage of dry weight below 3% were discarded from the statistical analysis. Components with a statistically relevant range of values were glucan (cellulose, hemicellulose, and soluble sugars), xylan (hemicellulose), lignin, protein, and structural inorganics. We derived soluble glucan by subtracting structural glucan from total glucan. Components with confirmable variation were then tested for linear correlation against other constituents. We also tested the effect of irrigation, planting density, and variety on a representative subset of the population, eight samples of 33P67 and eight samples of B73xMO17, using full factorial 2³ statistical analysis.

In this study, many methods were used to identify outliers in the population. Initially, outliers were identified by global-H and neighborhood-H values representing the closeness of each sample to the family of samples in the model. The standard deviation of values within this sample set was also calculated. Samples with values above or below two standard deviations (95% confidence interval) were noted. To further elucidate outliers of our 61-sample population and isolate components having the greatest effect, we performed the chi-square test on all eight significant constituents. Then, by excluding components from the chi-square test and observing the change in goodness-of-fit, we could determine the component(s) most responsible for a sample's outlying character.

Results

The FOSS NIR spectrometer (see Table 1) was used to predict twelve common corn stover constituents. The range and standard deviation within this data set for each of these constituents are shown in Tables 1 and 2. Only the fractions - total glucan, structural glucan, soluble glucan, lignin, protein, arabinan and structural inorganics - ranged greater than +/-1.5%, the accuracy of the wet chemical methods used to produce the model. Soluble glucan ranged 13.8% (see Table 2). Acetyl, uronic acid, galactan, mannan and soil values ranged less than 3% and were excluded from further analysis.

Additionally, no significant linear correlation of any two of the twelve compositional components could be found.

The standard deviation between duplicates was under 1% for all but one sample, 2868-091. This sample was shipped in two boxes and milled separately, labeled 2a and 2b. These separate boxes of sample 2868-091 had a 3% variation in total glucan, 18% in xylan and 20% difference in lignin. However, duplicate grabs from 2a and 2b respectively yielded an expected standard deviation below 1% (see Table 3).

Of 61 samples, none had global-H or neighborhood-H values above the maximum of 3. However, the nine samples of 33P67 from field two had outlying neighborhood H values above one (Table 1). Average mass closure for all samples was 97.39%.

Full factorial 2³ analysis showed that irrigation, planting density, and variety had no effect on the variation of relevant constituents in Pioneer varieties Mo17xB73 and 33P67. (Table 4). The error of the method is +/-1.5% dry matter, so any effect less than 3% could not be considered significant. Soil-free and soil-and-structural-inorganic-free analysis yielded similar non-significant results.

Of the exotic varieties, *Teosinte parviglumis* (Plot 17) showed the most striking difference in composition (see Figures 1 and 2). *T. parviglumis* had total glucan and soluble glucan (Table 1) levels more than two standard deviations above the population mean, high protein levels, a xylan fraction more than two standard deviations below the mean, and significantly lower lignin. Chi-square analysis for all eight significantly varying constituents (more than 3%) produced a value of 0.002. The same analysis omitting total glucan (i.e., soluble sugars) yielded a value of 0.20 when all other chi-square values were raised to over 0.90 without soluble sugar (see Table 5). The neighbor-H average of the *Teosinte* was 0.8; the global-H was 1.2. All the other exotics tested, including *Teosinte* crosses, showed no significant difference in composition.

Discussion and Conclusions

In this study, the components that varied the most - total glucan, structural glucan, and soluble sugars - may play the greatest role in conversion economics. While total and structural glucan represent cellulosic material to be degraded first into sugar and then to ethanol, soluble sugars may represent "free" sugar, in that pretreatment and

saccharification are not needed to make these soluble sugars available for fermentation. It may even be possible to wash these sugars from the corn stover for the production of enzyme needed for simultaneous sacharification and fermentation (SSF). For these reasons, identifying what causes or prevents high levels of soluble sugars could play an important role in optimizing ethanol production from corn stover.

From this data set, two examples showed abnormally high levels of soluble sugars. In field one, Pioneer 33P67 has soluble sugar levels around 7%. However, for some unknown reason, soluble sugar levels about twice as high were found in field two for the same variety under very similar growing conditions (see Figure 1). It seems likely that harvest variation might cause such a difference. As corn finishes its annual life cycle it probably metabolizes most available sugars in the process of dying. However, stover harvested near the time of grain harvest, while still green, its phloem still rich in photosynthetically produced soluble sugars, would likely still contain these sugars preserved in the drying oven. Further investigation is recommended to test the effect of post-harvest standing time in the field and time till drying after harvest on total glucan and soluble sugar content. Other factors, such as rain on exposed stover that may wash away sugars, may play a role in lowering soluble sugar content.

There are many possible explanations of the disparity in composition between Plot 2A and 2B (see Table 3). Corn stover varies in composition by anatomical fraction. Shipped and milled separately, these divisions of the same plot may have been divided unequally,

possibly a different ratio of anatomical fractions occurring in each box. Though, until more information is obtained from Ken Russell on this plot and other unknowns between fields one and two, there is no absolute explanation for the disparity in composition between Plot 2A and 2B.

In the case of *T. parviglumis*, speculation could be made to the contrary. Chi-square analysis of Pioneer 33P67 on a soluble-sugar-free basis showed that its other constituents are normal, whereas, the same analysis shows that *T. parviglumis* has a statistically distinct composition with greater difference than simply in high soluble sugars (see Table 5). With about 10% more soluble sugar available for fermentation that the other samples and significantly lower lignin a greater understanding of the cell-wall structure of T. parviglumis could aid in breeding a better stover. Through phloroglucinal staining of lignin rich xylem tissue in the vasculature, we might be able to visualize lower lignin levels. Additionally, because other crossed lines of Teosinte and commercial corn parentage showed normal composition, the testing of individual plants and the selfing of these crosses may segregate recessive traits that raise glucan or lower lignin levels. Other variables to consider are anatomical differences such as nodal length and ontogenetic stage in development at the time of harvest. T. parviglumis. was harvested after the same number of days as the other plots in the study but may have been at a different developmental stage

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Tables

TABLE 1. University of Nebraska Corn Stover Compositional Data (61 Samples)

Color Key

Above Two Standard Deviations

Near Two Standard Deviations High

Near Two Standard Deviations Low

Below Two Standard Deviations

Neighbor-H > 1.0	
Field One Plots	
Field Two Plots	

	4-4-1	-44	1 - 1 - 1 -						Dist	Halada Halada	Density Hi=3,	Lunday a 41 a va
Sample			soluble glucan	xylan	lignin	protein	ara- binan	structinorg	Plot #	Hybrid/ Inbred Designation	Med=2, Low=1	Irrigation (in./week)
·												
2868-066	44.0	37.8	6.2	22.0	18.5	2.1	3.0	1.4	1	B73 x Mo17	1	0
2000 004	42.7	35.9	6.8	20.5	17.2	3.0	2.7	3.5	2	D70v Ma47	4	0
2868-091	42.7	35.9	0.8	20.5	17.2	3.0	2.1	3.5	2	B73x Mo17	1	0
2868-100	41.1	34.2	6.9	19.6	15.7	3.9	2.5	4.9	3	B73 x Mo17	3	0
2868-076	42.0	35.4	6.6	20.2	16.9	3.3	2.8	3.8	4	B73 x Mo17	3	0
2868-061	43.4	37.6	5.8	20.6	18.1	2.8	2.3	2.0	5	B73 x Mo 17	1	1.5"
2000-001	40.4	37.0	5.0	20.0	10.1	2.0	2.0	2.0	3	B/3 X WO 17	ı	1.5
2868-094	42.1	35.5	6.6	20.7	16.3	3.1	2.8	3.9	6	B73 x Mo17	1	1.5"
2868-080	40.1	33.0	7.1	19.2	15.9	4.4	2.6	5.3	7	B73 x Mo17	3	1.5"
2868-089	38.8	32.1	6.6	18.7	15.8	5.2	2.7	5.9	8	B73 x Mo17	3	1.5"
2000-009	30.0	JZ. I	0.0	10.7	13.0	J.Z	۷.1	J.8	J	DIOXIVIOII	J	1.0
2868-086	42.4	35.8	6.7	22.1	16.4	2.7	2.9	3.4	9	Pioneer 33P67	1	0

	total	atruat	soluble				oro		Plot	Hybrid/ Inbred	Planting Density Hi=3, Med=2,	Irrigation
Sample				xylan	lignin	protein	ara- binan	structinorg		Designation	Low=1	inch./week
2868-087	42.6	36.1	6.5	21.6	16.2	2.4	2.9	4.3	10	Pioneer 33P67	1	0
2868-075	43.6	37.5	6.0	21.1	17.6	2.2	2.5	3.2	11	Pioneer 33P67	3	0
2868-062	42.5	35.7	6.8	21.0	16.1	2.6	2.6	5.0	12	Pioneer 33P67	3	0
2868-073	42.0	34.6	7.4	22.1	15.3	3.4	2.5	3.8	13	Pioneer 33P67	1	1.5"
2868-081	41.9	34.4	7.6	21.0	14.7	3.5	2.5	5.1	14	Pioneer 33P67	1	1.5"
2868-059	43.1	36.1	6.9	22.3	16.5	2.7	2.4	3.3	15	Pioneer 33P67	3	1.5"
2868-095	42.7	35.6	7.1	22.3	16.2	2.8	2.6	3.7	16	Pioneer 33P67	3	1.5"
2798-069	41.7	33.2	8.6	20.4	15.1	3.1	2.5	5.9	17	Tehua	unknown	1.5"
2868-070	50.3	30.7	19.5	14.5	12.2	4.5	-1.1	4.7	18	Teosinte	unknown	1.5"
2868-064	40.7	33.0	7.7	20.8	15.3	3.8	2.9	4.9	19	Cornbelt x Brazilian pop.	unknown	1.5"
2868-079	42.6	35.4	7.2	20.4	16.0	3.1	2.5	4.2	20	Cornbelt x Mexican pop.	unknown	1.5"
2868-071	40.8	33.5	7.2	21.0	16.1	3.7	2.8	4.7	21	Cornbelt2 x Teosinte	unknown	1.5"
2868-063	42.0	33.6	8.4	20.3	14.7	3.6	2.5	5.2	22	Early cornbelt pop.	unknown	1.5"

Density Hi=3, Hybrid/ Inbred Med=2, total struct soluble Plot ara-Irrigation Sample glucan glucan xylan lignin protein binan structinorg # Designation Low=1 inch./week 2.9 2868-099 43.5 35.1 8.3 21.0 14.8 2.3 3.9 23 W Synthetic 1.5" unknown 40.1 24 2868-068 31.4 8.7 19.5 14.8 4.8 2.4 5.9 NS(RFS) C9 unknown 1.5" 14.2 2868-088 41.7 31.7 10.0 20.1 4.3 2.2 5.1 25 NB(SI) C9 unknown 1.5" 2868-074 40.6 32.1 8.5 20.7 14.8 4.0 2.6 5.7 26 1.5" Midland (S) unknown 33.4 2.5 2868-085 40.7 7.3 19.5 15.3 4.0 5.8 27 Leaming (s) C5 unknown 1.5" 2868-084 41.2 33.1 8.1 20.9 14.4 3.4 3.0 5.4 Hoegemeyer 2641 unknown 1.5" Hoegemeyer 40.8 33.2 29 2868-092 7.5 22.5 15.1 3.2 2.4 4.8 2641 1.5" unknown CHIS775: N1912)-14.8 2868-096 40.5 32.1 8.4 19.1 4.7 2.0 6.0 31 1.5" 14 unknown CHIS775: N1912) 2868-077 41.8 33.3 8.5 18.7 16.2 4.5 1.7 5.5 32 unknown 1.5" -14 ARO30506: N09)-32.2 2.9 33 2868-060 40.3 8.1 21.3 15.1 4.0 5.1 1.5" 12 unknown AR030506: N09)-2868-093 19.8 16.8 40.8 33.8 6.9 2.4 34 unknown 1.5" 4.1 5.1 12 2868-065 40.2 33.2 7.0 20.9 15.4 4.2 2.6 5.0 35 FS8A: S09)-6 unknown 1.5" 3.0 2868-090 40.8 34.2 6.6 22.1 16.6 3.5 3.7 36 FS8A: S09)-6 1.5" unknown

Density Hi=3, Hybrid/ Inbred Med=2, total struct soluble Plot ara-Irrigation Sample glucan glucan xylan lignin protein binan structinorg # Designation Low=1 inch./week DREP150:N2012)-37 39.0 30.9 8.1 18.3 14.5 5.1 2.4 7.4 1.5" 2868-057 24 unknown DREP 150: 38.7 30.7 7.9 5.3 2868-058 19.3 14.6 2.6 6.8 38 N2012)-24 unknown 1.5" CHIS740: 2868-082 40.9 33.8 7.1 19.7 15.5 3.7 2.7 5.1 39 S1411a) unknown 1.5" CHIS740: 2868-098 40.5 33.0 7.5 20.8 14.5 3.8 3.1 4.9 40 S1411a)-7 1.5" unknown 32.5 2868-083 40.0 7.5 18.9 15.5 4.5 2.6 5.3 41 B73 unknown 1.5" CHIS 740: 2868-078 39.4 32.1 7.3 20.6 16.1 4.4 3.1 4.0 42 S1411a) 1.5" unknown 32.5 2868-097 39.9 7.3 22.7 15.5 3.7 3.4 3.9 43 1.5" Mo17 unknown 2868-072 39.8 32.5 7.3 22.4 15.8 3.8 3.4 3.9 44 1.5" Mo17 unknown 2798-071 44.2 30.9 13.3 20.4 12.9 3.6 1.3 5.2 33P67 3 1.5" 29.3 2798-061 42.4 13.1 19.7 11.7 4.4 1.7 7.2 33P67 1 1.5" 43.2 30.3 13.0 20.9 12.5 2 1.5" 2798-060 3.8 1.9 5.6 33P67 2798-072 45.1 30.4 14.7 20.0 12.3 3.7 1.3 5.0 33P67 1 1.5" 2798-062 45.7 31.0 14.6 18.7 11.6 3.6 8.0 6.0 33P67 2 1.5"

Density Hi=3, total struct soluble Plot Hybrid/ Inbred Med=2, Irrigation ara-Sample glucan glucan xylan lignin protein binan structinorg # Designation Low=1 inch./week 30.1 12.1 21.6 12.6 3.8 2798-067 42.2 2.0 6.0 33P67 3 1.5" 45.3 30.9 14.4 11.6 2798-074 20.0 3.6 1.1 5.5 33P67 2 1.5" 2868-073 44.6 12.8 20.7 13.0 31.8 3.3 1.2 5.4 33P67 3 1.5" 2798-070 45.1 29.6 15.5 18.7 11.5 4.1 0.9 5.8 33P67 1 1.5" 1.5" 40.6 31.6 9.0 2.8 2798-068 21.1 14.5 3.7 6.0 B73/mo17 1 33.5 14.7 2798-065 41.8 8.3 20.6 3.0 2.5 6.1 B73/mo17 2 1.5" 41.1 32.4 8.7 6.2 2798-059 20.7 14.9 3.5 2.6 B73/mo17 3 1.5" 2798-075 40.7 31.7 9.1 20.2 13.8 3.8 2.4 7.2 B73xMo17 1.5" 1 2798-069 41.7 33.2 8.6 20.4 15.1 3.1 2.5 5.9 B73xmo17 3 1.5" 32.9 8.2 19.9 2.3 1.5" 2798-064 41.1 14.8 3.5 6.5 B73xmo17 2 41.0 2798-063 32.5 20.5 14.9 2.5 1.5" 8.5 3.6 6.0 B73xmo17 3 2798-066 41.6 33.9 7.7 19.9 15.4 3.2 2.2 6.1 B73xmo17 3 1.5" 3.9 31.7 9.4 2.3 6.7 2891-069 41.0 19.9 14.0 B73xMo17 1 1.5"

TABLE 2. Summary of compositional data for each of 12 predicted constituents.

5 1		<u>-</u>		
	Maximum	Minimum	Range	Average
total glucan	50.3	38.7	11.6	41.8
structural glucan	37.8	29.3	8.4	33.1
soluble sugars	19.5	5.8	13.8	8.7
xylan	22.7	14.5	8.2	20.4
lignin	18.5	11.5	6.9	15.0
structural inorganic	7.4	1.4	6.0	5.1
protein	5.3	2.1	3.3	3.7
arabinan	3.4	-1.1	4.5	2.4
acetyl	3.0	0.9	2.1	2.3
uronic acids	3.5	1.4	2.1	2.8
galactan	2.1	-0.4	2.5	1.6
mannan	1.7	0.7	1.0	0.9
soil	1.5	1.2	0.3	1.4
Global H	2.6	0.7	1.9	1.3
Neighbor H	1.5	0.2	1.3	8.0
Mass Closure	101.1	90.0	11.1	97.4

TABLE 3. Difference in composition between Plot 2A and Plot 2B, fractions of the same plot of corn representing the same genetic line, field/growing conditions, and harvest.

				June		
Scanning date	June 25th	July 2nd	July 2nd	25th	July 2nd	July 2nd
Plot	Plot 2A	Plot 2A	Plot 2A	Plot 2B	Plot 2B	Plot 2B
Sample #	2868-091	2868-091	2868-091	2868-091	2868-091	2868-091
total glucan	45.03	44.63	44.68	40.31	39.95	39.88
structural						
glucan	38.90	38.45	38.49	32.81	32.71	32.24
xylan	22.15	22.02	21.75	18.88	19.21	18.99
lignin	18.62	18.42	18.89	15.69	15.97	15.22
protein	1.65	1.81	1.92	4.36	4.50	4.50
acetyl	2.59	2.73	2.73	2.58	2.53	2.58
uronic acids	3.35	3.36	3.29	2.68	2.70	2.67
galactan	1.85	1.89	1.86	1.84	1.94	1.89
arabinan	2.75	2.89	2.80	2.73	2.81	2.84
mannan	0.82	0.82	0.82	0.75	0.77	0.78
structural inorg	1.50	1.45	1.22	5.43	5.06	5.62
soil	1.28	1.26	1.27	1.36	1.34	1.33

Standard	Standard	Standard
Deviation	Deviation	Deviation
June 25th	Plot 2A(all)	Plot 2B(all)
3.34	0.22	0.23
4.30	0.25	0.31
2.31	0.21	0.17
2.07	0.24	0.38
1.92	0.14	0.08
0.01	0.08	0.03
0.47	0.04	0.02
0.01	0.02	0.05
0.01	0.07	0.06
0.05	0.00	0.01
2.78	0.15	0.28
0.05	0.01	0.01





Plot 2A Plot 2B

.

TABLE 4. Total Square Analysis Example. (Total Glucan, Plots 1-16)

									1
			Planting			VI			
Total G	Blucan	Variety	Density	Irrigation	VD		DI	VDI	
	44.0	1	-1	-1	-1	-1	1	1	KEY
	42.7	1	-1	-1	-1	-1	1	1	No Irrigation = -1
	41.1	1	1	-1	1	-1	-1	-1	Irrigation = 1
	42.0	1	1	-1	1	-1	-1	-1	High Planting Density = 1
	43.4	1	-1	1	-1	1	-1	-1	Low Planting Density = -1
	42.1	1	-1	1	-1	1	-1	-1	B73xMo17 = 1
	40.1	1	1	1	1	1	1	1	PIONEER 33P67 = -1
	38.8	1	1	1	1	1	1	1	
	42.4	-1	-1	-1	1	1	1	-1	
	42.6	-1	-1	-1	1	1	1	-1	
	43.6	-1	1	-1	-1	1	-1	1	
	42.5	-1	1	-1	-1	1	-1	1	
	42.0	-1	-1	1	1	-1	-1	1	
	41.9	-1	-1	1	1	-1	-1	1	
	43.1	-1	1	1	-1	-1	1	-1	
	42.7	-1	1	1	-1	-1	1	-1	
SUM		0	0	0	0	0	0	0	
plus		41.8	41.3	41.7	41.6	42.5	42.0	41.9	
minus		42.6	42.9	42.5	42.8	41.9	42.3	42.5	
effect		0.9	1.6	0.8	1.1	0.6	0.2	0.6	

TABLE 5. Chi-square analysis of *Teosinte parviglumis* against 61 samples. Column one represents chi-square of all 8 significantly varying constituents, while column two shows the chi-square results calculated on a soluble sugar free basis.

	Chi-	Square	Analysis
Sample #	Soluble Sugar	Without Soluble Sugar	
2798-069	0.003	0.20	teosinte p.
2868-086	0.97	1.00	Field One
2868-087	0.98	1.00	Pioneer 33P67
2868-075	0.89	1.00	
2868-062	1.00	1.00	
2868-073	1.00	1.00	
2868-081	1.00	1.00	
2868-059	0.97	1.00	
2868-095	0.99	1.00	
2798-071	0.84	1.00	Field Two
2798-061	0.70	0.99	Pioneer 33P67
2798-060	0.89	1.00	
2798-072	0.60	1.00	
2798-062	0.49	0.96	
2798-067	0.94	1.00	
2798-074	0.60	0.99	
2868-073	0.89	1.00	
2798-070	0.36	0.97	

Figures

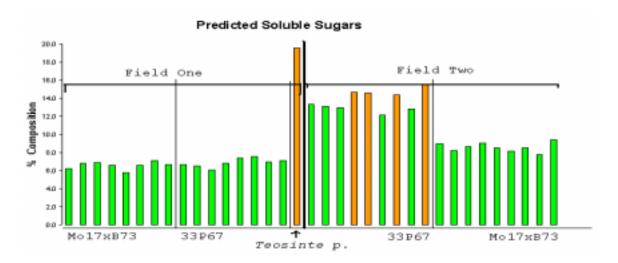


FIGURE. 1. Noticeable variation in soluble sugar content in identical lines 33P67 and outstanding soluble sugar content of *Teosinte parviglumis*.

FIGURE. 2. Comparison of composition of 60 Zea mays samples and Teosinte parviglumis.

